Characterization of Brain Phenotype in Unfixed, Refrigerated Transgenic Mouse Pups with Tissue-Specific FGFR3 Mutation by MR Microimaging

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Introduction

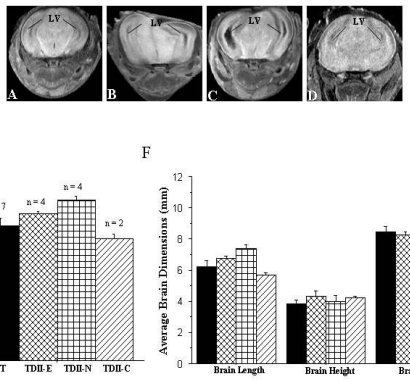
Thanatophoric dysplasia type II (TDII) is a lethal birth defect characterized by gross skeletal abnormalities, including a "cloverleaf" skull, as well as bilateral bulging of the temporal lobes and a small, underdeveloped cerebellum (1,2). TDII is known to arise from a mutation in the second tyrosine kinase domain of the fibroblast growth factor receptor FGFR3 (3). Since mutated FGFR3 may be present throughout the developing fetus, it is unclear whether the observed brain abnormalities in TDII patients are a direct result of abnormal FGFR3 in developing neural tissue or whether they are an indirect result of malformations in the cranium and spine which alter normal cereberospinal fluid flow and pressure. To address this question specifically and generally to examine the role of fibroblast growth factor receptors in normal and pathological central nervous system (CNS) development, we have expressed the mutation resulting in human TDII in transgenic mice. Using tissue specific promoters and the *Cre-loxP* system, we have bred mice in which the FGFR3 mutation occurs globally (TDII-E), in CNS tissue only (TDII-N) or in cartilage only (TDII-C). The resulting homozygous mouse pups die soon after birth and so must be immediately preserved for detailed study of brain morphology. To rapidly characterize the CNS abnormalities in these pups, we have employed diffusion-weighted MR microimaging post mortem. Pups were refrigerated during scanning using a simple, MR-compatible cold air source, eliminating the technical difficulty of perfusion fixing with paraformaldehyde and the distortions in tissue morphology which may occur with fixation.

Methods

Immediately after death, each mouse pup was frozen and stored at –20° C. Just prior to imaging, the pup was thawed, tied to a wooden tongue depressor, placed in a 20 mm glass NMR tube and inserted into using a Bruker DMX microimaging spectrometer equipped with a Magnex 9.4 T superwidebore magnet and actively-shielded gradients. The mouse pup was maintained at a temperature of +4°C during scanning by blowing cold air supplied from a vortex tube (Exair, Cincinnati, OH, USA) through the variable temperature apparatus of the Bruker Micro2.5 RF probehead. Since the vortex tube requires no electricity, refrigerant or moving parts to operate and consumes only room temperature compressed air, refrigeration of the neonate could be maintained indefinitely with no operator intervention or danger of magnetic or electromagnetic interference with the scanner. Diffusion-weighted spin echo MRI images were acquired of the brain in axial, sagittal and coronal planes using a diffusion gradient strength of 250 mT/m, duration δ=5 ms and separation Δ=11.5 ms. All images were acquired with TR = 5 s and TE = 23.2 ms. Typically, images were acquired with a matrix size of 512 X 256 pixels, field of view 2 X 2 cm or 4 X 2 cm and slice thickness 0.5 mm, resulting in volumetric resolution of 39 X 78 X 500 µm (axial slices) or 78 X 78 X 500 µm. For each slice orientation, total scan time was either 1.4 h (with four averages) or 2.8 h (with eight averages). For each pup, slices were selected with matching anatomical landmarks and were scaled uniformly for visual comparison. Total brain volumes were calculated by manually tracing the exterior edges of brain tissue in 30-40 contiguous axial slices and summing the areas contained within these boundaries.

Results

Representative axial diffusion-weighted images of the brains of wild type and TDII mouse pups are shown below. In these images "LV" indicates the lateral ventricles of the brain. Panel A shows a typical wild-type control while panels B-D show images of TDII-E, TDII-N and TDII-C transgenic mouse pup brains, respectively. It is immediately evident that in the TDII-E and TDII-N mice, the lateral ventricles are grossly enlarged and asymmetric while the TDII-C mice showed few morphological differences from wild type mice. These qualitative observations are reflected in the brain volume data shown in panel E. Here, no significant difference was seen between wild type and TDII-C while TDII-N and TDII-E mice showed significantly greater total brain volume than controls ($P < 0.0008$). These differences in brain volume corresponded to significantly larger brain width and length in the TDII-E and TDII-N pups (panel F), while no significant differences in overall brain height were found.



In addition to these data, comparison of sagittal images between the four groups of mouse pups showed dramatically smaller cerebella in TDII-E and TDII-N pups relative to TDII-C or wild type controls.

Discussion

Both qualitative comparisons of brain images and quantitative measurements of brain dimensions and volume consistently showed that wild type and TDII-C mouse brains were similar in ventricle size and symmetry, overall size and cerebellar appearance while mice with TDII-N and TDII-E tissue-specific mutations showed severe brain deformations similar to those reported in human TDII patients. Because mice of the TDII-N group underwent prenatal development with mutated FGFR3 only in CNS tissue and yet demonstrated profoundly abnormal brain size and shape, we conclude that expression of this mutation in CNS alone is sufficient to cause TDII-like brain malformation. Conversely, mice with mutated FGFR3 only in cartilage develop gross skeletal abnormalities in the spine but do not have enlarged lateral ventricles or increased brain size relative to wild type controls. From these observations, we conclude that mutation of FGFR3 in CNS tissue is both necessary and sufficient to create brain abnormalities in transgenic mice analogous to those reported in human TDII neonates. Finally, we note that refrigeration using the MR-compatible vortex tube is sufficient to preserve unfixed mouse pups during long high-resolution microimaging experiments.

References

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